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Sublethal concentrations of ichthyotoxic alga *Prymnesium parvum* affect rainbow trout susceptibility to viral haemorrhagic septicaemia virus

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ABSTRACT: Ichthyotoxic algal blooms are normally considered a threat to maricultured fish only when blooms reach lethal cell concentrations. The degree to which sublethal algal concentrations challenge the health of the fish during blooms is practically unknown. In this study, we analysed whether sublethal concentrations of the ichthyotoxic alga *Prymnesium parvum* affect the susceptibility of rainbow trout *Oncorhynchus mykiss* to viral haemorrhagic septicaemia virus (VHSV). During exposure to sublethal algal concentrations, the fish increased production of mucus on their gills. When fish were exposed to the algae for 12 h prior to the addition of virus, a marginal decrease in the susceptibility to VHSV was observed compared to fish exposed to VHSV without algae. If virus and algae were added simultaneously, inclusion of the algae increased mortality by 50% compared to fish exposed to virus only, depending on the experimental setup. We concluded that depending on the local exposure conditions, sublethal concentrations of *P. parvum* could affect susceptibility of fish to infectious agents such as VHSV.

KEY WORDS: Viral haemorrhagic septicaemia virus · VHSV · *Oncorhynchus mykiss* · Fish disease · Harmful algal blooms · Susceptibility

INTRODUCTION

When culturing rainbow trout in marine areas in Denmark, the fish are typically transferred from freshwater to marine facilities in the early spring and harvested in late autumn of the same year. During offshore mariculture, there is no possibility for controlling the water quality. Environmental conditions are very important for the performance of the fish and can significantly influence disease outbreaks in fish farms. Ichthyotoxic microalgal blooms represent one such environmental risk factor in marine aquaculture (Landsberg 2002). However, little is known about whether ichthyotoxic blooms can affect the occurrence and/or the severity of outbreaks of infectious fish diseases.

One of the most significant diseases in European aquaculture is viral haemorrhagic septicaemia (VHS; Olesen 1998), which is caused by viral haemorrhagic septicaemia virus (VHSV). Rainbow trout *Oncorhynchus mykiss* is the farmed fish species most susceptible to VHS, and during disease outbreaks, fish show clinical signs including lethargy, darkening of the body, pale gills and exophthalmia; upon necropsy, haemorrhages are seen in internal organs, musculature, meninges and eyes. Mortality of adult fish in fish farms is usually between 30 and 70% (Skall et al. 2005).

Marine rainbow trout farms in Denmark have experienced several VHSV outbreaks since 1982 (Jørgensen 1992, Dale et al. 2009). Some of the earlier outbreaks were due to transfer of virus-infected

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fish from freshwater facilities (Hørlyck et al. 1984), but intensive efforts to eradicate VHSV (Olesen 1998) from freshwater farms in Denmark have successfully eliminated this infection route (Bang Jensen et al. 2014). However, VHSV is also widespread in the marine environment and has been isolated from a range of wild fish species in both the North Sea (Smail 2000, King et al. 2001) and the Baltic Sea (Mortensen et al. 1999, Gadd et al. 2011). The virus has so far been found throughout the northern hemisphere, including the USA, Canada and Japan, and the number of recorded wild host species is still increasing as a result of increased monitoring (Skall et al. 2005).

Generally, the genotypes of VHSV from the marine environment exhibit low virulence to rainbow trout (Skall et al. 2004). However, there are several recent indications of direct transfer of VHSV of intermediate virulence from marine wild fish to marine farmed rainbow trout. In Finland, VHSV was isolated from 4 marine rainbow trout farms in 2000, and in 2004, the virus had spread to 24 farms in 3 separate locations. Results comparing gene sequences of the Finnish VHSV strains support the hypothesis that wild fish populations were the source of the primary infection (Raja-Halli et al. 2006). Similarly, the infection route was also assumed to be from wild fish during outbreaks of VHS in marine rainbow trout farms in Sweden during 1998 and 2000 (Skall et al. 2005) and in Norway during 2007 (Dale et al. 2009).

In addition to infectious diseases, blooms of ichthyotoxic microalgae can also result in mass mortalities of aquatic organisms, including fish in mariculture (Landsberg 2002, MacKenzie et al. 2011). Fish in closed aquaculture structures such as net cages have higher risk of being affected by harmful algal blooms, as they cannot escape from the bloom area. During sublethal blooms of ichthyotoxic microalgae, fish may not display any visual signs of being affected. Albright et al. (1993) reported that the diatoms *Chaetoceros* spp., through physically harmful mechanisms, increased the susceptibility of salmonid fish to bacterial infections. Furthermore, other scientists have discussed the possibility that natural fish populations exposed to blooms of harmful microalgae might have higher risks of outbreaks of infectious diseases (Seymour 1980, Landsberg 1995, Noga 1998). With the exception of these few reports, the possible effect of sublethal algal concentrations on the susceptibility of fish to infectious diseases has received little attention and remains to be addressed experimentally.

The toxin-producing alga *Prymnesium parvum* is a considerable threat to aqua- and mariculture worldwide due to its ability to form ichthyotoxic blooms (Johnsen et al. 2010, Southard et al. 2010). The first *P. parvum* bloom to be recorded in a large marine area was in the Ryfylke fjords in Norway during 1989. The bloom started developing in low-salinity water around 4–5 psu, but extended out of the fjord and caused fish mortalities at salinities up to 27 psu. In total, the bloom was responsible for the death 750 t of farmed Atlantic salmon *Salmo salar* and rainbow trout (Kaartvedt et al. 1991, Edvardsen & Paasche 1998). There are numerous hypotheses regarding the ichthyotoxic effects of *Prymnesium* spp. Suggestions include increased permeability of fish gills resulting in disturbed ion balance (Ulitzur & Shilo 1966b, Edvardsen & Imai 2006) or possibly a neurotoxic substance (Schug et al. 2010). The responsible toxins, their chemical structure and mode of action are still debated, and the actual number of toxins involved in the toxic effects observed is still unknown (Manning & La Claire 2010). Prymnesin 1 and 2 (Igarashi et al. 1995, 1998, 1999) and various fatty acids (Henrikson et al. 2010, Bertin et al. 2012) have been suggested to be the responsible toxins associated with fish kills caused by *P. parvum*.

While the physiological effects of *P. parvum* on fish are not fully understood, we aimed to determine whether sublethal exposure to *P. parvum* would affect the susceptibility of fish to an important pathogenic virus. It is expected that concurrent exposure to ichthyotoxic algae and pathogenic microorganisms would occur regularly in the marine environment, and knowledge on how this affects the health of farmed fish will help to optimize farm management strategies to mitigate potential health risks.

MATERIALS AND METHODS

Algal culture conditions

Prymnesium parvum (Kalmar University Culture Collection, strain KAC 39) was grown in 10 l Pyrex® bottles at 10°C in 15 psu water (35 psu seawater diluted with tap water). After dilution, water was heated to 95°C for 90 min and subsequently cooled, before nutrients were added to make F/2 medium (Guillard & Ryther 1962). Diurnal cycle was 12:12 h light:dark, and light was provided by cool white fluorescent tubes at 120 µE m^{-2} s^{-1}, measured inside the algal culture bottle with a Li-Cor®, LI-1000 radiation sensor equipped with a spherical probe. Aeration

(~6 l h^{-1}) was provided by a Hiblow 40 air pump through a 5 cm spherical air stone. Algal cultures were inoculated to a starting concentration of 10 × 10^3 cells ml^{-1} and grown to a concentration of 0.8 × 10^6 to 1.0 × 10^6 cells ml^{-1} before use. All algal cultures used for experiments were in exponential growth phase.

VHSV

VHSV isolate DK-3592B originating from a clinical outbreak of VHS in farmed rainbow trout in Denmark was used at low passage number for all experiments (Lorenzen et al. 1993). BF-2 cells (Wolf et al. 1966) were used for propagation and titration of the virus. Virus for exposure of fish was in the form of cell culture supernatant harvested from VHSV-infected cell cultures showing complete cytopathic effect (CPE) as described earlier (Lorenzen et al. 2000). Titres were calculated as tissue culture infective doses of 50% ml^{-1} of sample ($TCID_{50}$) (Rovozzo & Burke 1973). Virological examination of dead fish was done by inoculation of homogenized tissue material on BF-2 cell cultures followed by a subsequent analysis of the cell culture supernatant using a VHSV-specific ELISA (Mortensen et al. 1999).

Analysis for anti-viral effect of the algae

Before exposing fish to *P. parvum* and VHSV, we examined whether the alga was able to inactivate or affect infectivity of the virus; comparative titration on cell cultures was undertaken on virus samples exposed to a single algal concentration for different time periods. Three tubes each containing 50 ml algal culture (49 × 10^3 cells ml^{-1}) and 1 tube containing 50 ml medium (control) were inoculated with 50 µl of VHSV (final titre 4.8 × 10^4 $TCID_{50}$ ml^{-1}). After 1, 4 and 7 h, subsamples were withdrawn for titration. Ten-fold dilution series were prepared and 25 µl inoculated in 12 replicate wells of 96-well microtitre plates containing BF-2 cell cultures. The plates were incubated at 15°C for 7 d, before microscopic examination for CPE and calculation of $TCID_{50}$.

Fish exposure trials

All trials were performed at a water temperature of 10°C and a salinity of 15 psu. Aeration was adjusted to keep oxygen saturation at 80%. The fish were acclimated to these conditions for at least 14 d prior to use. The fish were fed with a standard commercial fish feed (INICIO Plus 1.5 mm, Biomar) until 2 d before use. In all experiments, algal concentration, pH and O_2 were measured every 24 h. In all exposure trials, the fish were monitored regularly and frequently, and fish showing clinical signs of disease were registered and terminated by prolonged immersion in anaesthetic (0.01% benzocaine). At the end of the exposure trials, surviving fish were similarly terminated and counted. All animal procedures were in agreement with the EU Directive 2010/63/EU for animal experiments and performed with permission from the Danish Animal Experiments Inspectorate.

Algal exposure conditions

Before exposing fish to algae and virus, the conditions for sublethal exposure to *P. parvum* were optimized in terms of algal cell concentration. These experiments included 3 replicate 12 l glass aquaria. Ten litres of water with suspended algae were added to each aquarium followed by addition of 5 rainbow trout (10 g each). The dose response test included 8 concentrations of algae in the range 40 × 10^3 to 350 × 10^3 cell ml^{-1}. Exposure times were either 12 or 24 h, followed by a complete exchange of the algae-containing water with algae-free water.

Exposure of fish to *P. parvum* and VHSV

Three combined exposure trials were performed, all with rainbow trout fingerlings kept in replicate 8 l aquaria with 50% water renewal per day. Experiment (Expt) 1 included initial exposure to *P. parvum* followed by exposure to VHSV, while Expts 2 and 3 included simultaneous exposure to algae and virus. Exposure experiments were performed with 2 replicate aquaria in Expt 1, and 3 replicate aquaria in Expts 2 and 3. Results were analysed with the Kaplan-Meier Log-Rank Survival Test (SigmaPlot Version 11.0, Systat Software).

Pre-exposure to algae (Expt 1)

A single algal concentration was tested in combination with 2 different viral dilutions to measure the susceptibility of the fish to infection with VHSV. The fish were exposed to the algae for 12 h, followed by a

Table 1. Dose of viral haemorrhagic septicaemia virus (VHSV) and concentration of *Prymnesium parvum* in combined exposure experiments. Rainbow trout *Oncorhynchus mykiss* were exposed to 2 different viral doses in Expt 1, and 3 different doses in Expts 2 and 3. In Expt 1, fish were exposed to *P. parvum* for 12 h prior to introduction of the virus, whereas in Expts 2 and 3, the fish were exposed to both virus and algae from the start. Expts 2 and 3 differed in that Expt 3 used more (but smaller) fish than Expt 2. No significant difference was found between the sublethal algal concentrations within each of the 3 experiments. Fish mortality was not observed in the controls exposed to algae only (data not shown)

VHSV exposure dose ($TCID_{50}$ ml^{-1} aquarium water)	*P. parvum* (10^3 cells ml^{-1})	Percent mortality (replicates)
Expt 1		
7.4×10^4	0	96 (96, 96)
	57 ± 5	92 (96, 88)
5.0×10^2	0	76 (81, 71)
	59 ± 2	67 (72, 62)
Expt 2		
8.9×10^4	0	52 (67, 40, 50)
	73 ± 3	87 (100, 80, 80)
4.1×10^2	0	48 (38, 45, 60)
	77 ± 13	47 (80, 10, 50)
1.9×10^1	0	0 (0, 0, 0)
	67 ± 8	7 (20, 0, 0)
Expt 3		
4.1×10^4	0	89 (96, 83, 88)
	85 ± 6	78 (62, 87, 84)
5.0×10^3	0	70 (73, 78, 58)
	91 ± 2	70 (52, 76, 83)
6.0×10^2	0	61 (72, 50)
	92 ± 5	69 (64, 88, 56)

complete water renewal prior to addition of virus. After exposure to virus for 2 h, the water was renewed once again. The experiment was terminated after 32 d. Each aquarium contained 25 fish (5 g) and the setup consisted of 6 treatments in duplicate aquaria: (1) Negative control (cell culture medium only), (2) fish exposed to *P. parvum* and cell culture medium added subsequently, (3)–(6) fish exposed to 2 different viral doses with or without prior exposure to *P. parvum*. Algal concentrations and viral titres are listed in Table 1.

Simultaneous exposure to algae and virus (Expts 2 and 3)

In Expt 2, the fish were exposed to both VHSV and algae from the start of the experiment. After 24 h, all of the water was renewed. Three different viral doses were tested ($VHSV_{1\text{-}3}$), and the experiment comprised 8 different treatments including controls. Algal concentrations and viral titres are listed in Table 1. The experiment was performed in triplicate aquaria with 10 fish (12–15 g) in each aquarium and was stopped after 22 d.

As with Expt 2, Expt 3 also consisted of 8 treatments in triplicate aquaria, and the fish were exposed to VHSV and algae from the start of the experiment but with an increased number of fish included in each tank (25 fish, 7–8 g). Algal concentrations and viral titres are listed in Table 1. The experiment was stopped after 21 d.

RESULTS

Analysis for antiviral activity of the alga

The *Prymnesium parvum* culture did not affect the infectivity of the virus. We found no significant difference in viral titre between the control (virus only, 7.1×10^3 $TCID_{50}$ ml^{-1}) and the virus that had been incubated with *P. parvum* culture for 1, 4 or 7 h ([4.8, 4.0 and 8.6] $\times 10^3$ $TCID_{50}$ ml^{-1}, respectively; $p = 0.299$, Kruskal-Wallis 1-way ANOVA on ranks).

Determination of sublethal *P. parvum* concentrations

Initial exposure trials revealed that algal cell concentration in the range of 50×10^3 to 100×10^3 cells ml^{-1} was the highest range to be well tolerated by the fish, although an initial phase of gill mucus secretion was observed. In the subsequent exposure trials, we therefore aimed at this range. In pilot experiments, high aeration appeared to remove the ichthyotoxicity of *P. parvum* suspensions (data not shown); aeration was therefore kept at the minimal level required to maintain oxygen saturation above 80%.

Fish exposure to *P. parvum* and VHSV

In all 3 exposure experiments, the fish showed mucus production from their gills when exposed to sublethal algal concentrations. Following exposure to VHSV, dying fish developed typical clinical signs of VHS including exophthalmia, external haemorrhages particularly in the opercula and in the buccal cavity, darkening of the skin and uncoordinated swimming. These fish were euthanized. An overview of the cumulative mortalities at the end of the experi-

ments is given in Table 1. Data are presented as means with mortality of each replicate. Cumulative mortalities are depicted as a function of time (see Figs. 1–3). Data are presented as means ± SD.

Expt 1

In this experiment, the fish were exposed to algae for 12 h prior to the exposure to 2 different VHSV concentrations. The mortality rates of the fish treated with VHSV and VHSV + algae were not significantly different at either of the 2 viral dilutions (p = 0.515 and 0.438), although fish that were exposed to the algae before exposure to VHSV showed a tendency towards lower mortality compared to the group exposed to VHSV only (Fig. 1). No fish died in the control group exposed to cell culture medium only, nor in the group exposed to algae only.

There was no significant difference in the algal concentrations between the groups, viz. *P. parvum* ([57 ± 2] × 10^3 cells ml^{-1}), $VHSV_1$ + *P. parvum* and $VHSV_2$ + *P. parvum* (Kruskal-Wallis 1-way ANOVA on ranks, p = 0.933).

Expt 2

In this experiment, the fish were exposed to both algae and virus in combination from the start of the experiment. Three different VHSV concentrations were included. In the group exposed to the highest VHSV concentration, exposure to VHSV alone induced a cumulative mortality of 52%. This mortality increased significantly to 87% (p < 0.001) when the fish were exposed to both VHSV and algae (Fig. 2A). A similar difference at the end of the trial was not seen in the 2 groups exposed to the intermediate and the lowest viral concentrations (p = 0.708 and 0.168, respectively; Fig. 2B,C). However, a faster development of VHS was evident in the group given the intermediate virus dose with algae compared to the fish exposed to this viral dose only (Fig. 2B). Furthermore, there was an unexpected high variability between the mortalities in the replicate aquaria in this particular group. Except for 2 fish in the group exposed to both VHSV and algae, no mortality occurred in the treatments with the lowest VHSV concentration (Fig. 2C).

We found no significant difference in the algal concentrations between the groups (*P. parvum* at [54 ± 3] × 10^3 cells ml^{-1}, $VHSV_1$ + *P. parvum*, $VHSV_2$ + *P. parvum* and $VHSV_3$ + *P. parvum*, 1-way ANOVA, p = 0.155). The data from the group exposed to *P. parvum* only included 2 replicate aquaria instead of 3; fish in the third replicate aquarium showed darkening of the skin and highly increased mortality from the start of the experiment and data from that aquarium were therefore excluded from the results.

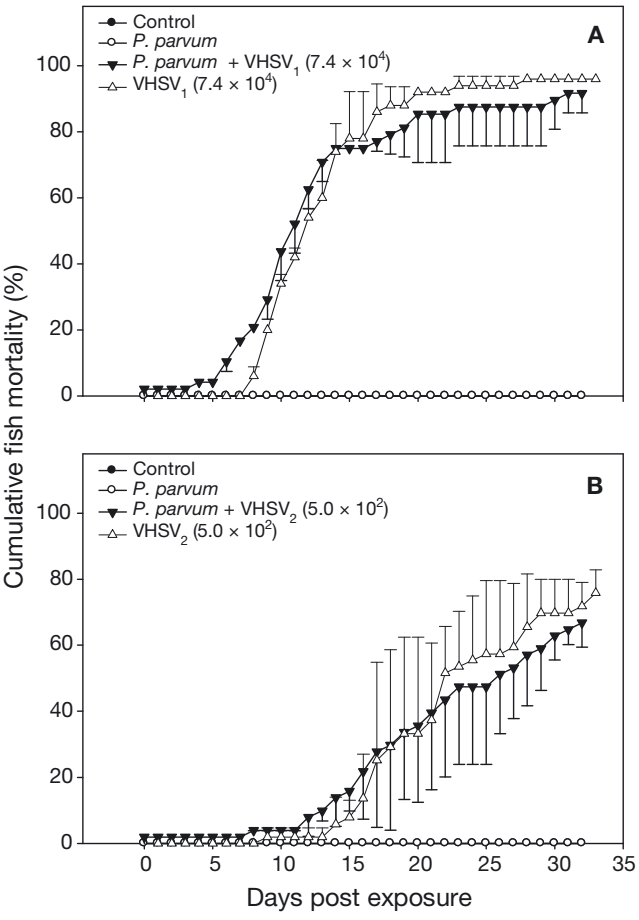

Fig. 1. Cumulative mortality (mean ± SD) of rainbow trout *Oncorhynchus mykiss* (n = 25) when pre-exposed to the harmful alga *Prymnesium parvum* 12 h prior to exposure to viral haemorrhagic septicaemia virus (VHSV) at 2 different concentrations (Expt 1): (A) 7.4 × 10^4 and (B) 5.0 × 10^2 $TCID_{50}$ ml^{-1}. Open triangles: mortality of fish exposed to VHSV only; closed triangles: mortality of fish exposed to both VHSV and *P. parvum*. There was no mortality in the control fish or in the fish exposed to a sublethal concentration of *P. parvum* only (closed and open circles, respectively)

Expt 3

As in Expt 2, fish in this experiment were exposed to both VHSV and algae from the start of the experiment. The fish were approximately half the size of

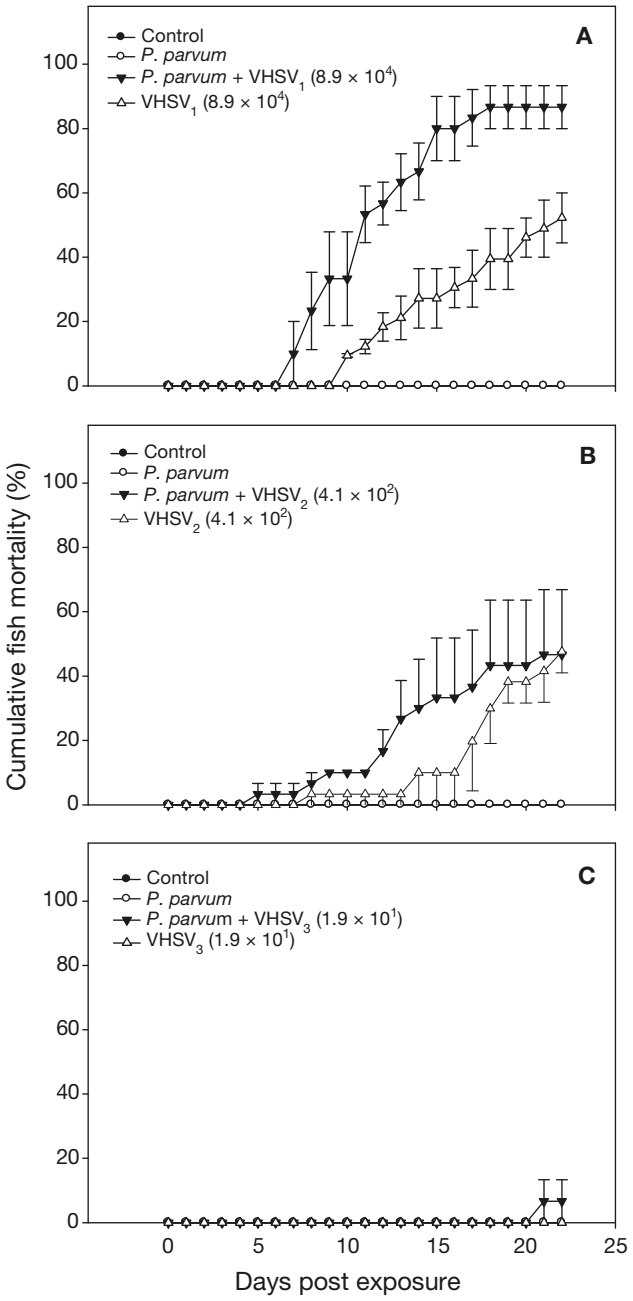

Fig. 2. Cumulative mortality (mean ± SD) of rainbow trout *Oncorhynchus mykiss* (n = 10, 12–15 g) when simultaneously exposed to *Prymnesium parvum* and viral haemorrhagic septicaemia virus (VHSV) at 3 concentrations (Expt 2): (A) 8.9×10^4, (B) 4.1×10^2 and (C) 1.9×10^1 $TCID_{50}$ ml^{-1}. Symbols as in Fig. 1

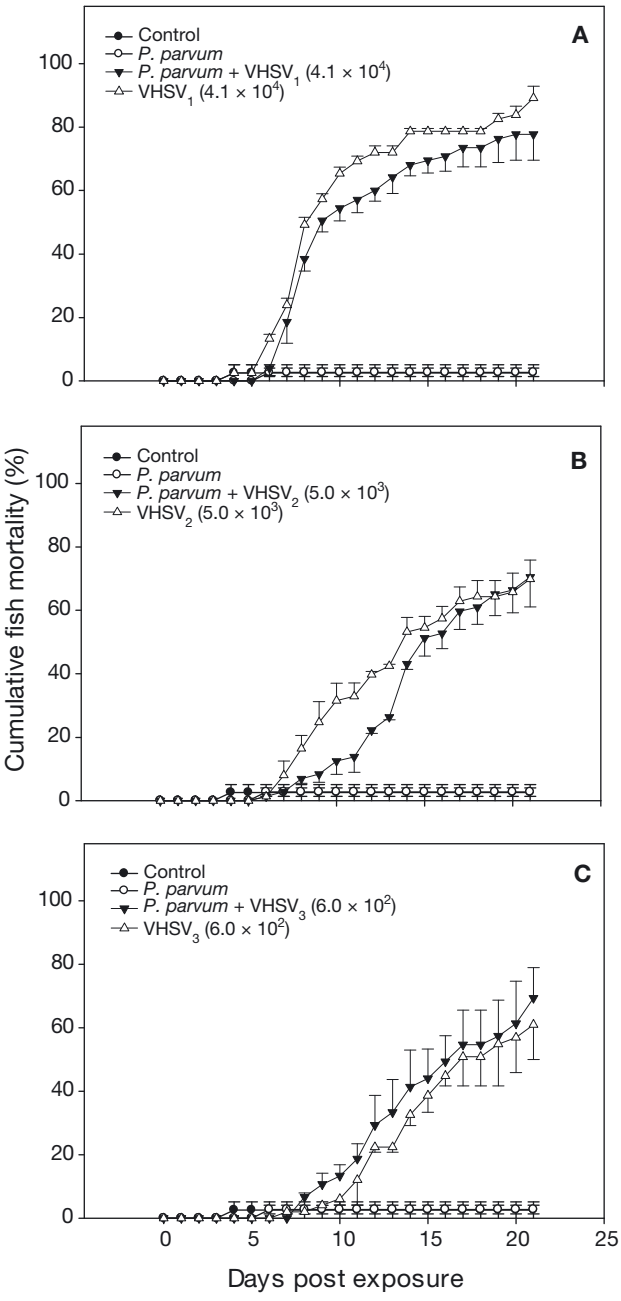

Fig. 3. Cumulative mortality (mean ± SD) of rainbow trout *Oncorhynchus mykiss* (n = 25, 7–8 g) when simultaneously exposed to *Prymnesium parvum* and viral haemorrhagic septicaemia virus (VHSV) at 3 concentrations (Expt 3): (A) 4.1×10^4, (B) 5.0×10^3 and (C) 6.0×10^2 $TCID_{50}$ ml^{-1}. Symbols as in Fig. 1

the fish used in Expt 2, and the highest concentration of VHSV induced acute and high mortality (78–89%; Fig. 3), which was not significantly dependent on simultaneous exposure to algae ($p = 0.071$). In the fish given the low and intermediate viral doses, mortality was also rather high (61–70%) and was independent of algae ($p = 0.596$ and 0.478). The dataset for the fish exposed to the low viral dose without algae consisted of only 2 replicates, since no mortality was observed in 1 of the triplicate aquaria and

subsequent virological examination showed no virus in the fish. This aquarium was therefore excluded from the data set.

There was no significant difference in the algal concentrations between the groups (*P. parvum* at [94 ± 12] × 10^3 cells ml^{-1}, $VHSV_1$ + *P. parvum,* $VHSV_2$ + *P. parvum*, $VHSV_3$ + *P. parvum*, 1-way ANOVA, p = 0.695).

DISCUSSION

This study is the first of its kind that attempted to determine whether prior or simultaneous exposure of fish to an ichthyotoxic alga affects the susceptibility of the fish to a viral pathogen. Our findings reveal that sublethal exposure of rainbow trout to the haptophyte *Prymnesium parvum* under certain conditions can exacerbate the disease caused by infection with the rhabdovirus VHSV.

Precisely how *Prymnesium parvum* affects fish is still not well understood. From laboratory tests and natural blooms, it is known that *P. parvum* affects a broad range of teleost fish (Shilo 1971, Lindholm et al. 1999), including rainbow trout (Kaartvedt et al. 1991), the species used in the present study. In our experiments, we used whole algal cultures only, but cell-free supernatant (Shilo & Aschner 1953) and extracted intracellular toxins (Shilo & Rosenberger 1960) can also kill fish. In fact, Ulitzur & Shilo (1966a) demonstrated that gills are highly affected by extracted intracellular toxins.

We only observed the toxic effects on rainbow trout caused by *P. parvum* itself within the first 24 h of exposure (data not shown). For that reason, fish were not exposed to algae/algae + virus for more than 24 h in the performed experiments. The algal concentration was intentionally adjusted to a sublethal level to allow analysis of the combined effect of VHSV and algae. During all of our experiments, mucus secreted into the opercular cavity of the fish was clinically observed when they were exposed to concentrations of *P. parvum,* suggesting that the algae indeed affected the gills.

When fish are exposed to VHSV in a water bath, all body surfaces of the fish are exposed to the virus. For a long time, the gills were considered the main portal of entry for VHSV into the fish, since the gills were the first organ where the virus could be demonstrated post infection (Neukirch 1984). It might thus be hypothesized that sublethal algal concentrations could further predispose this transmission route through the gills. Later, other transmission routes were identified, through skin and fin bases (Harmache et al. 2006, Montero et al. 2011) as well as *per os* (Schönherz et al. 2012). Comparative histological studies on the propagation of VHSV in fish exposed to different concentrations of *P. parvum* are required to determine how this alga affects development of VHS as reported here.

When fish were exposed to *P. parvum* for 12 h prior to exposure to VHSV, a slightly lower mortality was observed in the group exposed to both VHSV and *P. parvum* compared with the group exposed to VHSV only (Fig. 1). Whether this might be explained by the presence of secreted mucus or physiological effects such as reduced blood flow in capillaries in exterior tissues reducing the uptake of virus requires further analyses to be clarified. We also cannot exclude the possibility that algal exposures upregulate innate defense mechanisms, and thereby temporarily affect the susceptibility to viral disease.

In our 2 experiments with combined exposure to algae and virus, fish sizes and numbers were different due to adjustment of fish density according to the available size of fish. In Expt 2, we thus used 10 fish of 12–15 g per 8 l aquarium, compared to 25 fish of 7–8 g in Expt 3. The smaller fish used in the latter trial displayed higher and more acute mortality following exposure to virus by itself compared with the larger fish in Expt 2. A significant effect of *P. parvum* on the outcome of VHSV exposure was only evident in Expt 2, suggesting that a viral challenge which by itself induced intermediate mortality could be influenced more by *P. parvum* compared with a VHSV challenge mediating high mortality by itself. The high variability between replicate aquaria exclusively in the group of *P. parvum*-exposed fish receiving an intermediate viral dose in Expt 2 further suggested that under certain conditions, minor environmental variations (e.g. airflow/oxygen saturation) could have a significant effect on the outcome of combined algal and viral exposure. It is also possible that the increased gill/biological surface area of the fish in Expt 3 might have reduced the effect of the algae. Earlier reports have thus shown that the amount of the target material affects the toxicity of a *P. parvum* cell suspension to target organisms (Reich & Rotberg 1958, Tillmann 2003).

The present study demonstrates that the experimental setup can influence the results when performing fish trials that involve combined exposure to sublethal concentrations of ichthyotoxic algae and infectious agents like VHSV. Further studies are required to determine which combinations of fish, algal and viral exposure result in the most detrimen-

tal effects. Nevertheless, the results presented here stress the importance of taking local environmental parameters into account in evaluating the risks of infectious disease outbreaks in marine aquaculture. VHSV is prevalent in the marine environment (Skall et al. 2005), and although marine isolates usually display no or low pathogenicity to rainbow trout, simultaneous exposure to toxic algae could increase the chance of VHS outbreaks in sea-reared rainbow trout. Similarly, since *P. parvum* affects several fish species (Shilo 1971), and marine VHSV isolates are pathogenic to several marine fish species (Mortensen et al. 1999), combined exposures might also affect both wild and cultured populations of these species. Vaccination can efficiently prevent VHS (Lorenzen & LaPatra 2005), and future studies are planned to evaluate whether vaccination against VHS can also prevent disease when the fish are simultaneously exposed to toxic algae.

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